ECOLOGICAL FACTORS AND DROSOPHILA SPECIATION

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INTRODUCTION

In 1927 there appeared H. J. Muller's announcement of the artificial transmutation of the gene. This discovery was received with enthusiasm throughout the scientific world. Ever since the days of Darwin biological alchemists had tried in vain to induce those seemingly rare alterations in genes which were coming to be known as "the building stones of evolution." In the same year Charles Elton published a short book on animal ecology. It was received with little acclaim. That is not surprising. To the modern biologist ecology has seemed a bit out-moded, rather beneath the dignity of a laboratory scientist. Without detracting from the importance of Muller's discovery, in the light of the developments of the past 13 years we venture to say that Elton comes nearer to providing the key to the process of evolution than does radiation genetics.

Here is a quotation from Elton's chapter on ecology and evolution. "Many animals periodically undergo rapid increase with practically no checks at all. In fact the struggle for existence sometimes tends to disappear almost entirely. During the expansion in numbers from a minimum, almost every animal survives, or at any rate a very high proportion of them do so, and an immeasurably larger number survives than when the population remains constant. If therefore a heritable variation were to occur in the small nucleus of animals left at a minimum of numbers, it would spread very quickly and automatically, so that a very large porportion of numbers of individuals would possess it when the species had regained its normal numbers. In this way it would be possible for non-adaptive (indifferent) characters to spread in the population, and we should have a partial explanation of the puzzling facts about closely allied species, and of the existence of so many apparently non-adaptive characters in animals. . . . Finally what little we know about the regulation of numbers in animals enables us to say that the problem of the origin of species can only be successfully solved by the aid of work on numbers."

Two extreme views of the dynamics of evolution appear almost equally inadequate. Among paleontologists it has seemed popular to consider the evolving species as represented by one plastic individual projected indefinitely through time, molded into new form by the direct action of the surrounding environment. In contrast the theory of natural selection sets forth the premise that evolution is brought about by the action of selective factors on a population indefinitely large and in breeding equilibrium. Actually in any species there occur populations, finite in size, fluctuating possibly in rhythm with some climatic factor, and also fluctuating arhythmically in size in response to variable arhythmic ecological factors and combinations thereof. In fact the general rule is irregularity and non-predictable variability. Thus the rise and fall of gene frequencies and the fixation or elimination of certain types are determined or conditioned by a set of variables that differ both in space and in time.

SPECIATION IN DROSOPHILA

To many biologists Drosophila means a small yellow fruit-fly, the species melanogaster. This, indeed, is the most famous of the lot, and used far more than any other for the investigation of genetic and cytological problems. However, the genus contains a great many other forms. From published descriptions and correspondence with collectors I have recently made out a list (certainly not complete) of 72 distinct types of Drosophila collected within the boundaries of the U. S. A. Over half of these we have had in culture in the laboratory at Wooster. Harrison Stalker and I have taken 26 species in Ohio.

One of the hoary arguments of the anti-evolutionist has been that never are new species seen to arise from old ones. By defining species as that group of individuals which can breed and produce fertile offspring the argument was properly sewed up in a neat scrap of fundamentalist logic. If, however, an array of cases can be presented within a group such as Drosophila, and shown to be seriated in regard to degrees of incompatibility between parental stocks and sterility of the hybrids, the case seems to be almost as strong for the occurrence of speciation as if one might sit quietly by and watch the whole business. And some study of the situation leads to the impression that probably the only reason we don't see the process is that we are too impatient to sit that long, or too undiscerning to see what is happening.

An examination of the genus indicates that not all species are equally dissimilar in morphological and physiological characters. Sturtevant (1939) has recently made a thorough study of the living species then available to him (over 40 in all) and on the basis of 27 characters for which each form was analyzed has proposed three sub-genera. Within these sub-genera sections are recognized. Thus the genus falls into groups of what appear to be more or less closely related species. At present immunological studies are projected or in progress in certain laboratories as a further check on these relationships. Although Drosophila of many species had been worked on much earlier, five years ago hybrid crosses had been secured only in the cases of simulans by melanogaster, pseudo-obscura A by B, and both races of pseudo-obscura by miranda. Two years ago in addition azteca had been crossed to athabasca, virilis virilis to virilis americana, and affinis affinis to affinis iroquois, seven cases in all. Today 20 cases of hybridization between diverse types are known in this genus.

Theoretically it would seem possible to seriate the differences or divergences between pairs of Drosophila types, so that it could be stated that these two were closely similar, a second pair more distinct, and a third even less alike, and so on. Actually in Drosophila an attempt may be made to seriate in terms of morphology, physiology and behavior, sexual reactions, gonial chromosomes, salivary chromosomes, geographical distribution, etc. A man-made scheme would no doubt see to it that all fell neatly into the same order of seriation under the several criteria mentioned. Actually by no stretch of the investigator's best scientific imagination can this be done. A little reflection may lead to the conclusion that this is a normal state of affairs if the course of evolution is actually determined by a complex of interacting forces.

Following Dobzhansky's definition of species as that stage of the evolutionary process, "At which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of interbreeding," we might attempt to arrange a series with regard to inter-type incompatibility and hybrid sterility. Difficulties in the way of applying the above definition of species and in seriation in this respect will appear presently. Not only do inter-group incompatibility and hybrid sterility, both factors in the physiological isolation called for by Dobzhansky's definition, frequently if not generally differ fundamentally in their causes and operation, but there is no uniform rule in regard to their relative strength in various cases. Further, there is sometimes great variation in the incompatibility of several strains of form A with one strain of form B, though all the strains of form A are highly compatible inter-se. Both incompatibility and hybrid sterility, but particularly the former, are highly susceptible to culture conditions. Darwin (1859) in his chapter on hybridism puts the case most clearly in these words, "It is certain on the one hand, that the sterility of various species when crossed is so different in degree and graduates away so insensibly, and, on the other hand, that the fertility of pure species is so easily affected by various circumstances, that for all practical purposes it is most difficult to say where perfect fertility ends and sterility begins. I think no better evidence of this can be required than that the two most experienced observers that ever lived, namely Kölreuter and Gärtner, arrived at diametrically opposite conclusions in regard to some of the very same forms. It is also most instructive to compare the evidence advanced by our best botanists on the question whether certain doubtful forms should be ranked as species or varieties, with the evidence from fertility adduced by different hybridizers, or by the same observer from experiments made during different years. It can thus be shown that neither sterility nor fertility affords any certain distinction between species and varieties." This quotation is given not because it is from Darwin, but because it states the case with a clarity to be looked for in vain in the writings of some modern students of this subject. Quantitative tables giving in per cents the sterility, incompatibility, or fecundity of first crosses and hybrids in Drosophila speciation studies have appeared and will continue to appear (the author is guilty of having perpetrated some of them; Spencer 1940a). They give a false impression of accuracy in the quantitative measurements of factors which are actually extremely susceptible to fluctuations in the environmental set-up and to hereditary variations difficult of analysis by which several strains of a given form may differ.

Through recent hybridization studies which have been reviewed elsewhere (Spencer, 1940b), it is becoming increasingly clear that this genus contains a vast array of recognizably distinct forms, some distantly and others closely related. However, as we seem to approach an analysis of the problems of phylogeny and to be in a position to arrange forms according to their natural affinities, disturbing questions arise. Which are the more nearly related, two stocks which have for a long time period been separated by some effectual isolating mechanism and yet which show little difference in morphology and physiology, or two forms which have more recently diverged but have undergone rapid changes in that part of the genotype which conditions morphological and physiological differences easily recognized? Are two forms living under conditions where the total metabolism of the species is low to be considered more closely related than two forms which live in the tropics even though both pairs have been effectively isolated for the same length of time? In fact the closer one approaches an analysis the less certain does an exact objective description appear possible. It would seem that the dynamic pattern of evolution projected through time could not possibly be accurately described in terms of the static pattern of evolution in space. Perhaps the sooner we realize that we are chasing a will-o'-the-wisp in attempting to arrange the forms of life in a static hierarchy of phylogenetic relationships the better. However, in the light of recent progress I should judge that we are on the verge of finding out much more about evolution than we have known. The taxonomist will continue to have an important, in many cases the most important, role to play in facilitating the study of groups of animal and plant organisms.

It appears that the changes which result in evolution are primarily changes which occur in the structure of the chromatin. A point which may still be considered debatable in some circles is that these changes are discrete and sudden at their primary level, though slow cumulative effects may accrue by gradual and progressive increase in the number of these inherited differences in two diverging stocks. It is unnecessary here to review the variety of processes, structures and characters in living organisms which may be changed by mutation. There is evidence that mutant factors may alter the whole pattern of events in connection with mitosis, may condition increased mutability at other loci, or may favor loss or addition of whole chromosomes. We shall not enter into the controversy as to whether so-called gene mutations are inherently different from small chromosome aberrations. Certainly, and what seems more important, mutation itself may produce slight or profound effects on an organism, may involve one or many so-called loci, may in short provide all sorts of changes which in turn may be combined to give an almost incomprehensible array of permutations. These may conceivably interact with the internal and external milieu in ways innumerable.

That characters by which hybridizing forms of Drosophila differ may be monogenic has already been observed. Pupa case color in Drosophila virilis virilis is gray and in virilis americana is red. A single genetic factor is involved. On the other hand it is not surprising that many of the inter-subspecific differences are multi-factorial. There is no reason at present to believe that any of the characters morphological, physiological, or psychological by which two forms of Drosophila differ are determined by any inherently different mechanism than that which conditions those mutations with which the geneticist has been dealing for some years.

If this thesis be accepted it becomes possible to examine Drosophila populations for differences in gene and chromosomal structure and to consider such differences if found as the basic pattern for the first step in evolutionary divergence. If it be possible to find populations in which single gene or chromosome variants have become fixed it is valid to assume that the case is not inherently different from that in which an ensemble of genetic differences has accumulated and become fixed. We may go further and consider variations in frequency of unfixed genes in different populations to be an earlier and necessary step in evolution. It should be kept in mind that often the mutants with which we work may play a less important role in the development of new types than some which are less conspicuous but more fundamental in the sense that they may be acting to establish an isolating mechanism or to favor the group in natural selection when this becomes a directing factor.

ECOLOGY AND POPULATION MECHANICS IN DROSOPHILA HYDEI

Now, it is high time we get into the field. But being laboratory biologists suppose we shift over to field work by easy stages. Maybe we can learn something in the interim, and at least we can keep from getting our feet wet the first day. I want, therefore, to introduce you to *Drosophila hydei*. We shall go where this beast lives, watch it feed and breed, oviposit, overcome difficulties of temperature and moisture fluctuations. We shall see the eggs dry up one day, others hatch the next day. We shall see masses of semi-liquid medium shimmering in the noon-day sun with the writhing bodies of many thousands of tiny larvae. We shall see older larvae working down below the surface of medium now beginning to dry and later pupating in huge masses in still drier conditions. We shall see hydei die overnight by the tens of thousands, and leave a cold gray and lifeless world in a week or so where once there were literally a million insects breeding and feeding to the limit of the food store. We shall follow a few survivors through perilous days and weeks and months, and see them drop off one by one. We shall finally find the hardier ones and the luckier ones again breeding and see their grandchildren by the million; stark tragedy; the arrows of outrageous fortune; rare good luck; a perfect environment and for a time unlimited expansion with all the checks off; sizzling heat and stiffening cold; larvae drowned out and others dried up and still others coming through, surviving by strange coincidence a whole series of untoward events. This is everyday life for *Drosophila hydei* in any town in the northern part of our United States, and in any town worthy the name there hydei will be.

Drosophila hydei is a tropical species; there is some probability that it originated in Mexico. At least many closely related forms are to be found there. The species has worked northwards through the United States and is now well established in all parts of this country, but always breeding in and about towns and dwelling houses unless temporarily carried out into the open country with garbage or other refuse. In northern latitudes each town forms a potential focus for an "island" population. Such populations pass through several overlapping generations a year with tremendous fluctuations in population size. Even in the mildest winters in this latitude hydei does not overwinter out of doors. The only survivors are those which find refuge in houses, fruit-cellars, restaurants and such places at the time of the first heavy winter freeze. I have followed the course of the hydei population in Wooster for some years. Every month of the winter this fly may be seen in certain restaurants; I have also known it to overwinter in cellars of private homes. It is a robust type, and can live in a dry room, sitting about on the walls for most of the time, provided every day or so it has access to food and moisture for a few minutes. Such an invironment is provided in restaurants; even though there is no opportunity for a winter brood of larvae the adults may carry through from autumn to spring. I have kept adults sufficiently long to be certain of this. In fact the overwintering population is generally in the adult stage. In general the size of a town or village will determine the chance of suitable places for overwintering and of these places being populated at the critical time in the autumn.

Among Drosophila species hydei is not a rapid breeder, the minimum cycle from egg-laying to newly emerged adult being 14 days. To this must be added a minimum of two days for maturing of the female and four days for maturing of the male after eclosion. Thus the life cycle is 18 days for males and 16 days for females under food and temperature conditions optimum for development. Actually the cycle under spring and summer conditions with the diurnal temperature rhythm is in the neighborhood of one month to six weeks.

At this season of the year (May), even though stores of food are available only a few scattered specimens of hydei will be found outdoors. By the middle of June to the first of July thriving colonies of hydei may be observed on garbage heaps, but still the maximum populations for available food are not present. Toward late August and early September the populations will often have built up to a maximum. However, July and August are likely to be hot and dry, and stores of food unless concentrated in large heaps may dry up before a generation of hydei comes through. In Ohio the months of September, October, and November generally see the peak populations of this species built up; particularly is this true during good fruit years, and with moderate autumn precipitation keeping food supply at the proper moisture content. With the heavy freezes generally occurring in late November and early December the hydei population rapidly falls off. Sometimes the species has bred up to such enormous numbers in town that it may be trapped in nearby woods. But this is due to population pressure and not because hydei is capable of establishing itself in natural woodland habitat.

Drosophila hydei is capable of withstanding extremely high temperatures in larval, pupal and adult stages. On a large citrus dump in southern California, containing tons of decaying oranges, grapefruit and lemons, and populated by an adult Drosophila population of many millions, I made a study of temperature tolerance. It had been supposed that a temperature of 30° C., applied even for a few hours, would sterilize male Drosophila. On this citrus dump millions of adult *Drosophila hydei* were surviving with the air temperature a few inches above the surface registering 37° C. for several hours in the heat of the day. It was difficult to determine the exact temperature of the microenvironment forming the three or four millimeter film at the moist surface where the flies were gathered. A peculiar hovering reaction was noted under these conditions. Flies when disturbed flew with extreme rapidity but always in a dense swarm only a few inches above the moist medium and settled back on it quickly. At times the temperature of the medium in which larvae were burrowing was 36° and 37° C. Here many larvae were killed but others survived and when taken into the laboratory pupated normally; the flies emerging were normal and fertile. Thus larvae were observed in great numbers at temperatures at which well over half of them were killed; day after day the survivors had been subjected to these sublethal temperature shocks. I have kept hydei in adult, larval and pupal stages in a room above my garage in Wooster where mid-day temperatures went up to 36° C. *Drosophila hydei* may be the dominant form in summer populations in towns because of its ability to withstand intense heat. However, all species can stand higher temperatures than was formerly supposed. The lethal and sterilizing effects of high temperatures on Drosophila have been due not so much to the direct effect of the temperature as to the gases, particularly carbon dioxide, formed in poorly ventilated culture bottles.

Hydei larvae are also capable of withstanding drying up of food medium, at which time the larvae form small masses resulting in less surface loss of water. If then moist medium is again provided the larvae continue to develop although practically dormant during the drying period. The population pattern of *Drosophila hydei* brought about through the seasonal variations in temperature and moisture, with superimposed temporary and local fluctuations in food supply, moisture, competition with other forms, and temperature supplies the basis for the formation of local races or populations differing from others in the frequency of contained genes.

In a comprehensive series of papers on the mechanics of evolution in Mendelian populations Wright (1931; 1932; 1937) has presented a statistical analysis of the rate of evolution under various hypothetical conditions. For statistical purposes it has been necessary to reduce situations to their simplest terms, far simpler in fact then natural conditions warrant. Wright (1931) states briefly the role which fluctuating conditions may play in the following terms, "A question which requires consideration is the effect of alternation of conditions, large and small size of population, severe and low selection. The effects of changes in the conditions of selection have already been touched upon. Persistence of small numbers or of severe selection for such periods of time as to bring about extensive fixation of factors compromises evolution for a long time following, there being no escape from fixation except by mutation pressure. Many thousands of generations may be required after restoration to large size and not too severe selection, before evolutionary plasticity is restored. Short time oscillations in population number or severity of selection, on the other hand, probably tend to speed up evolutionary change by causing minor changes in gene frequency."

It is possible to make a census of the genetic structure of Drosophila populations for a given year by taking a sample of the population and through inbreeding tests analyzing the genetic variability of the flies. The author (Spencer 1939) has attempted a fragmentary analysis of this sort for two widely separated *Drosophila hydei* populations, one living on a large citrus dump near Azusa, Southern California, and the other in the environs of Wooster, Ohio. The Azusa population is much the larger, perhaps at peak one hundred times the maximum Wooster population. The hot, dry summer at Azusa and the winter in Wooster constitute the critical reduction periods. The analysis has included the collection and examination of over 50,000 wild flies, and the rearing of over 5000 F_2 pair mating broods from some 1200 wild flies from the 1937, 1938, and 1939 Azusa populations, and from the 1937 and 1938 autumn Wooster populations. Inbreeding tests of 100 flies from Gatlinburg, Tennessee, have also been made. More than 180 cases of autosomal recessive mutants carried in wild flies have been found. In addition several sex-linked factors and autosomal dominants have been recorded.

The 1938 Wooster population was characterized by the high concentration of a small group of mutant genes, sex-linked vermilion in .48% of males collected, nicked wings in 4 out of 331 flies tested, gray body in 12 out of 500 flies, scarlet eye in 3 out of 500 flies, and rose eye in 3 out of 331 flies. The Azusa population showed no such high concentration of specific genes. On the other hand a significantly larger number of mutant loci per 100 flies tested were to be found from Azusa than from Wooster. Azusa, Gatlinburg, and Wooster populations have each given a different lot of mutant loci with little overlapping. The samples taken were inadequate to give an accurate quantitative picture of gene frequencies with the exception of vermilion; however, these qualitative results make it possible to plan experiments for adequate quantitative tests of yearly fluctuations in specific gene frequencies.

All over the United States there occur local populations of *Drosophila hydei*, varying in average size with the size of the towns and available food stores, undergoing periodic oscillations as well as arhythmic fluctuations due to ecological factors, and setting the stage for micro-evolution through rise and fall of gene frequencies. There seems little doubt but that here and there throughout this vast array of populations there occur cases of fixation of some mutant type. If this mutation should happen to initiate a partially isolating mechanism an insipient subspecies would be the result.

We have considered the case of *Drosophila hydei* in some detail because of the fact that its close association with man makes it possible to observe its activities more thoroughly the year round. These observations may serve in a way to bridge the gap between strictly laboratory and bona fide field work. We may think of a study of hydei populations as a large scale experiment carried on through the intervention of man but clarifying somewhat the problems to be encountered in studying species breeding in their natural range.

SOME ECOLOGICAL OBSERVATIONS ON OTHER DROSOPHILA SPECIES

If you have the time suppose we do some field collecting. It is the month of May. We go to the Providence mountains, a range in the Mojave desert of Southern California. We climb to the top of the ridge at mid-day. As the sun beats down on stunted pinon pine and bare rocks the temperature mounts well above 100° F. We pick up a club and break open a rotting specimen of *Echinocactus acanthodes*, the California barrel cactus. Inside is a great bowl of cactus soup, alive with Drosophila larvae. The adults sitting around on the inside of this bowl, little yellow fellows, turn out to be *mulleri mojavensis*, a subspecies which can cross with *mulleri mulleri* from Texas. Later we see as we drive along a desert road a belt of barrel cactus at elevations of around 3000 feet, a thin line of supplies but adequate to allow *mulleri mojavensis* to migrate all over the deserts from mountain range to mountain range. We go again up the arroyo, with not a drop of water in sight, in fact not a spring in the entire range. We set out traps in the late afternoon, and soon pseudo-obscura are flying in apparently from an environment as dry as tinder except for a little resin oozing from the pinons. Here and there is that spiny denizen of the desert, *Yucca mojavensis*. We kick over one of these tough plants and at the base find a mass of soft, moist fiber with mold growing in it. Where molds grow yeasts grow and there we find pupae which look suspiciously like those of pseudo-obscura.

We go some hundreds of miles away to Death Valley and drive up the desert floor of the valley during the afternoon. The thermometer registers 110° F. in the shade; but there is no shade. We camp for the night at Mesquite Springs; a clear cool spring running a quart or so of water a minute, a dozen screw-bean trees with gnarled and spreading branches, and plenty of fine soft sand. We hang our traps near the spring and with the aid of a flashlight visit them at night. Temperature readings show the thermometer creeping down toward 30° C., then lower and at around 27° C. the flies begin coming to the traps, first that yellow one we took in the Providence mountains, then a black, shiny one, then a stray pseudo-obscura or two. These last should be far away in the cool of the mountains at this time of year, but here they are. Perhaps they will live out the summer, more than likely not.

It is August and we are back in Ohio. We have set our traps in cool, wooded nooks along a branch of the Killbuck creek. We visit them in the early morning for even in Ohio it will be hot at midday and most of the Drosophila will have left the traps and found a cool spot at the warning of that rapid mid-morning temperature rise. At six or seven in the morning the flies are coming to the traps and we collect two or three thousand in a couple of hours. Back in the laboratory we check them over, the usual ones, perhaps 15 or 20 species. Here are a couple we ought to know, but they don't look quite right. We go to the species stocks and get out macrospina from Texas. The ones in our morning catch have the peculiar genitalia of macrospina but they are away off in eye color, body color and size. They turn out to be a new subspecies, breeding true to their peculiar character but crossing readily with Texas macrospina. We call them *macrospina ohioensis* (Spencer 1940b). Another morning we visit traps set in the swamps. This morning we go early, four-thirty, and we get our feet wet. But we get something else as well. Two new Ohio species; but are they species? They are morphologically distinct with many character differences, but they will cross and their hybrid offspring are partially fertile. They cross with less ease than the macrospinas and we are not sure whether to call them species or subspecies. Somehow we can't seem to think that it makes much difference. On our trips we have taken flies that feed on fungus and others that feed on bleeding trees. We found larvae of the new swamp species feeding on rotting stocks of the arrow-weed.

We might discuss population patterns of flies breeding on leaf mold and humus all over our native woods, of flies confined to swamp habitats, of those which apparently feed mostly on fungus. There is much we do not know of the habits, the distribution, the ecology of these and others. We have a notion that field studies will help to answer some of the problems of speciation in the group. In any case it's a good excuse to be outdoors.

ENVIRONMENTAL FACTORS AND MUTATION

We have considered the action of the ecological pattern on distribution of mutant factors present in populations, resulting in shifts in gene frequencies; sometimes the elimination of mutant genes; at other times their rapid spread and even fixation. But there is more than a remote possibility that ecological factors may play an active role in producing the mutations involved. For many years men attempted to find the key to mutation. Muller finally found one key in X-rays. But he and Mott-Smith (1930) soon demonstrated that short wave radiation played no major role in natural mutation. Others, notably Jollos (1934), Goldschmidt (1929), and Plough and Ives (1935) have shown that sub-lethal heat shocks increase mutation rate. Gottschewski (1934) also demonstrated mutation increase through cold temperature shock. In recent years experiments have been reported, particularly by Russian workers, which indicated a sensible rise in mutation frequency following various other treatments, chiefly chemical. In fact we seem now to be in possession of too many keys. At the present time we may say that we cannot yet be sure that they all fit the lock.

Day after day, week after week, Drosophila in nature are receiving temperature shocks many of which are of the order of magnitude of those used by investigators reporting increase in mutation rate. The possible combinations of shock which might result from fluctuation in temperature, moisture and other ecological factors may conceivably be playing a direct role in determining the rate at which natural mutations are occurring. In fact experiments on wild stocks of *Drosophila melanogaster*, summarily reported in manuscripts received from Russian workers and read at the 7th International Genetics Congress in Edinburgh last summer set forth this point of view in some detail. This position may seem to be less satisfactory than the over-simplified view that some one definite factor could be discovered as the cause of mutation. The more we learn of the nature of the organic world the less chance there seems to be to reduce biology to a system of simple generalizations.

BIBLIOGRAPHY

Darwin, Charles. 1859. On the origin of species by means of natural selection. London. 6th edition, D. Appleton and Co., New York. xxi+458 pp.

Dobzhansky, Th. 1937. Genetics and the origin of species. Columbia Univ. Press, New York. xvi+364 pp.

Elton, Charles. 1927. Animal ecology. Sidgwick and Jackson, Ltd., London. xvii+207 pp.

Goldschmidt, R. 1929. Experimentelle Mutation und das Problem der sogenannten Parallelinduktion. Biol. Zbl. 49: 437–448.

Gottschewski, G. 1934. Untersuchungen an *Drosophila melanogaster* über die Umstimmbarkeit des Phänotypus und Genotypus durch Temperatureinflüsse. Z. indukt. Abstamm. und VererbLehre. 67: 477–528.

Jollos, V. 1934. Inherited changes produced by heat-treatment in *Drosophila melanogaster*. Genetica 16: 476–494.

Muller, H. J. 1927. Artificial transmutation of the gene. Science 66: 84–87.

Muller, H. J. and **Mott-Smith, L. M.** 1930. Evidence that natural radioactivity is inadequate to explain the frequency of "natural" mutations. Proc. Nat. Acad. Sci. 16: 277–285.

Plough, H. H. and **Ives, P. T.** 1935. Induction of mutations by high temperature in Drosophila. Genetics 20: 42–69.

Spencer, W. P. 1939. On the genetic structure of *Drosophila hydei* populations. Genetics 24: 86–87.

1940a. Subspecies, hybrids and speciation in *Drosophila hydei* and *Drosophila virilis*. Amer. Nat. 74: 157–179.

1940b. Levels of divergence in Drosophila speciation. Amer. Nat. 74: 299–311.

Sturtevant, A. H. 1939. On the subdivision of the genus Drosophila. Proc. Nat. Acad. Sci. 25: 137–141.

Wright, Sewall. 1931. Evolution in Mendelian populations. Genetics 16: 97–159.

1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution. Proc. 6th Int. Congress of Genetics 1: 356–366.

1937. The distribution of gene frequencies in populations. Proc. Nat. Acad. Sci. 23: 307–320.